FB PCR Clean Up/Gel Extraction Kit

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For research use only

Sample: up to 100μl of PCR Product or 300 mg of Agarose Gel

Recovery: up to 95%

Introduction

The FB PCR Clean Up/Gel Extraction Kit provides a cost-effective system for fast and easy isolation of DNA fragments from PCR reactions, agarose gels, or enzymatic reactions. DNA fragments (100bp-10Kb) in specialized buffers are bound by the glass fiber matrix of the spin column (1, 2) while contaminants pass through the column. Impurities are efficiently washed away, and pure DNA is eluted with Tris buffer or water without phenol extraction or alcohol precipitation. DNA purified with the kits is suitable for any subsequent application, such as ligation and transformation, sequencing, restriction enzyme digestion, labeling, PCR, in vitro transcription, or microinjection. The entire procedure can be completed within 15-20 minutes.

Kit Contents

Catalog No.	DE0004	DE0100	DE0200	DE0300
DE Buffer	2 ml	60 ml	110 ml	80 ml×2
W1 Buffer	2 ml	45 ml	85 ml	125 ml
W2 Buffer (Add Ethanol)	300μl×2 (1.2 ml)×2	15 ml (60 ml)	25 ml (100 ml)	25 ml×2 (100ml)×2
EL Buffer	1 ml	10 ml	20 ml	30 ml
DE Columns	4 pcs	100 pcs	200 pcs	300 pcs
Collection Tubes	4 pcs	100 pcs	200 pcs	300 pcs

Quality Control

In accordance with FairBiotech's ISO- certified Total Quality Management System, the quality of the FB PCR Clean Up / Gel Extraction Kit is tested on a lot to lot basis to ensure consistent product quality.

Additional requirements

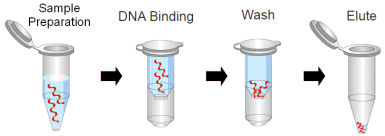
∗Ethanol (96~100%) ∗1.5 ml microcentrifuge tubes

NOTE

★ Add ethanol (96–100%) to Buffer W2, **shake before use** (see bottle label for volume).

★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.

★ Buffers DE and W1 contain irritants. Wear gloves when handling these buffers.



FB PCR Clean Up/Gel Extraction Kit Protocol

Step 1 Sample Preparation

Gel Extraction

- Excise the DNA fragment from the agarose gel. Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube. Add 500 μl **Buffer DE** to the sample and mix by vortex.
- Incubate at 60°C for 10 minutes (or until the gel slice has completely dissolved). During the incubation, mix by vortexing the tube every 2–3 minutes. Cool the dissolved sample mixture to room temperature.

PCR Clean Up

- Add 500 μl **Buffer DE** to 100 μl of the PCR reaction and mix by vortex.

Step 2 Binding

- Place a **DE Column** in a **Collection Tube**. Apply the supernatant (from step 1) to the **DE column** by decanting or pipetting.
- Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **DE column** back into the same **Collection tube.** (The maximum volume of the **DE column** reservoir is 800 μl. If the sample mixture is more than 800 μl, repeat the DNA Binding Step)

Step 3 Wash

- Add 400 μl of **Buffer W1** into the **DE Column**. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **DE column** back into the same **Collection tube.**
- Add 600 μl of **Buffer W2** (ethanol added) into the **DE Column**. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **DE column** back into the same **Collection tube.**
- Centrifuge at 14,000 x g again for 2 minutes to remove residual **Buffer W2**.

Step 4 Elution

- To elute DNA, place the **DE column** in a clean 1.5 ml microcentrifuge tube.
- Add 50-200 μl **Buffer EL** or water (pH is between 7.0 and 8.5) to the center of each **DE column**, let stand for 2 min, and centrifuge at 14,000 x g for 2 min.

Troubleshooting

Problem	Cause	Solution
Low yields of DNA	DE Buffer with the incorrect ratio added to the DNA product.	Verify that an correct volume of the DE Buffer was added to the reaction mixture.
	96~100% ethanol not used	Add ethanol (96~100%) to the Buffer W2 before use.
	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use new glass- and plastic-wares; wear gloves.
	Column overloaded	Decrease the loading volume. If overloaded , separate the reaction mixture into 2 columns. If the DNA fragments are more than 300mg, separate the gel slice into two microcentrifuge tubes.
	Dissolved incompletely	Increase time for the Gel Extraction Step until the gel slice has completely dissolved. Use an equal volume of the DE Buffer and/ or use low-melting-point agarose gels.
	Incorrect elution conditions	Ensure that the Buffer E or ddH_2Ois added into the center of the DE Column.
	Recovery buffer volume too small	Increase the amount of the EL Buffer to at least 50 μl for use.
Inhibition of downstream enzymatic reactions	TE buffer used for DNA elution	Use ethanol to precipitate the DNA, or repurify the DNA fragments and elute with nuclease-free water.
	Presence of residual ethanol in DNA	Remove the EtOH in the hood briefly. Following the Wash step, dry the DE Column with additional centrifugation at 14~16,000 x g for 2 minutes.
DNA passed through in the flow-through or wash fraction	Column overloaded	Check the loading volume. If overloaded , separate into two columns.
	Inappropriate salt or pH conditions in buffers	Ensure that any buffer prepared in the laboratory was prepared according to instructions.
Purified DNA floats out of wells while running in agarose gel	Traces of ethanol not completely removed from the column	Make sure that no residual ethanol remains in the membrane before eluting DNA. Re-centrifuge if necessary.